Table II. ^{14}C:^{3}H Ratio in Leu Derived from the Pepsin-Catalyzed Hydrolysis of [^{14}C] Leu-Tyr-[^{3}H] Leu[a]

% ^{14}C in Leu-Tyr-Leu substrate[b]	% ^{14}C in Leu product[b]	Hydrolytic susceptibility of bond 1:bond 2[c]
26.5[d]	50.2	2.3 ± 0.5
49.5[d]	64.3	
51.6[e]	67.9	2.4 ± 0.5
53.5[e]	76.6	

[a] For conditions, see Table I. [b] % ^{14}C is the number of ^{14}C counts as a percentage of the total counts. [c] Calculated from (^{14}C:^{3}H ratio in Leu)/(^{14}C:^{3}H ratio in Leu-Tyr-Leu). See Scheme I. [d] [Leu-Tyr-Leu] = 4 *μM:* transpeptidation is undetectable under these conditions. [e] [Leu-Tyr-Leu] = 60 *μM:* cf. the substrate concentrations used in the experiments reported in Table I. In these experiments, comparable amounts of Leu and of Leu-Leu were found on the amino acid analyzer.[9]

This possibility was eliminated by the finding that the ^{14}C:^{3}H ratio in the *N*-dansyl-Leu, derived from the N-terminal residue of the Leu-Leu product, was the same as in the *N*-dansyl-Leu derived from a previously hydrolyzed sample of the Leu-Leu product, within experimental error. The Leu-Leu predominantly derives, therefore, from the pathways outlined in Scheme I.[7]

The implications of these findings in the formulation of mechanistic proposals for the action of pepsin will be discussed fully elsewhere, but the requirement that transpeptidation products arise from both amino and acyl transfer suggests that after cleavage of the peptide link, *either* half of the substrate may leave first. Thus for such materials as Z-Tyr-Tyr,[2a] Z-Glu-Tyr,[2b] acetyl-Phe-Phe-Gly,[2c] and acetyl-Phe-Tyr,[2d,8] but not acetyl-Phe-Tyr-amide nor acetyl-Phe-Phe-ethyl ester,[2d] amino transfer has been observed. Yet, as reported here, transpeptidation from Leu-Tyr-Leu is predominantly (though not exclusively) via acyl transfer. While many earlier mechanistic proposals for pepsin that postulated an exclusive amino transfer pathway[1] now need some modification, it is probable that the relative importance of amino transfer and acyl transfer simply depends upon the ease with which the amino and acyl moieties of the cleaved substrate leave the active site.

Acknowledgment. We are grateful to the Science Research Council for support.

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(6) [^{14}C]Leu-Tyr-Leu and Leu-Tyr-[^{3}H]Leu were synthesized by classical methods. Boc-Tyr(OBzl) and Leu-OBzl were condensed with dicyclohexylcarbodiimide–*N*-hydroxybenztriazole–diethylisopropylamine, and the resulting dipeptide after removal of the Boc group with trifluoroacetic acid was coupled (as before) to Z-Leu, to give Z-Leu-Tyr(OBzl)-Leu-OBzl. Deprotection by hydrogenolysis provided Leu-Tyr-Leu. All intermediates gave satisfactory rotations and microanalyses. The radioactive samples—although pure by the normal criteria—were subjected to preparative high-pressure liquid chromatography before use. Leu-Tyr-Leu has $[\alpha]^{20}D$ −3.2° (*c* 1 in *N*-NaOH), mp 212° dec.

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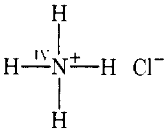
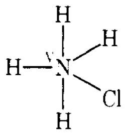
Onium Ions. XVI.[1] Hydrogen–Deuterium Exchange Accompanying the Cleavage of Ammonium (Tetradeuterioammonium) Trifluoroacetate by Lithium Deuteride (Hydride) Indicating SN2 Like Nucleophilic Displacement at Quaternary Nitrogen through Pentacoordinated NH_5

Sir:

A major stepping stone in the development of structural chemistry in the early part of the century was the realization of the ionic rather than covalent nature of ammonium compounds.[2] Ammonium compounds, as is well known, are tetravalent ionic (I), and not pentacovalent nitrogen compounds (II). It is, therefore, of substantial interest to report experimental evidence obtained in studies directed toward the investigation of ammonium hydride showing nucleophilic hydrogen exchange of the ammonium ion involving pentacoordinated NH_5. To our knowledge this study for the first time shows the pentacoordinating ability of nitrogen.



I



II

Our interest in NH_5 was aroused following successful studies in solution chemistry of five-coordinated CH_5^{+} [3] and its isoelectronic neutral boron analog, BH_5.[4] Both of these species have eight electrons surrounding the central atom involving two-electron three-center bonding. The ten-electron carbon analog (with a four-electron three-center bond) would be CH_5^{-} (or the fluorine analog CF_5^{-}) in a SN2 like reaction. Whereas nucleophilic substitution reactions at sp^3 carbon are well studied, no similar reactions were previously considered for tetrahedral nitrogen compounds (ammonium ions). We thought that the most obvious approach to inquire into the possibility of five-coordinated nitrogen pentahydride was to study the preparation and behavior of ammonium hydride, $^{+}NH_4H^{-}$. Whereas all other alkali hydrides are stable and well characterized, study of ammonium hydride has not been reported. Ammonium borohydride was studied in metathetic reactions and found unstable, liberating H_2[5] and forming the ammonia-borane complex.[6]

We considered that the most convenient way to approach the preparation of $NH_4^{+}H^{-}$, free of any solvent interference, was to carry out a metathetic reaction of a low melting ammonium salt with an alkali hydride. We, therefore, prepared ammonium trifluoroacetate ($^{+}NH_4{}^{-}O_2CCF_3$, mp 130°), and allowed it to react with lithium hydride in the melt. H_2 and NH_3 are formed besides lithium trifluoroacetate in the cleavage of the ammonium ion by the hydride through ammonium hydride (eq 1). The reactions were carried out in glass vessels, kept in an oven and flame dried

$$^{+}NH_4{}^{-}O_2CCF_3 + LiH \longrightarrow LiO_2CCF_3 + [^{+}NH_4{}^{-}H] \longrightarrow NH_3 + H_2 \quad (1)$$

prior to use, then attached to a vacuum line provided with suitable receiver vessels. After flushing with dry N_2, the system was evacuated to 5×10^{-3} mm and the reaction vessel heated at 130° until gas evolution ceased (10–20 min). The gaseous products were analyzed by mass spectrometry (Consolidated Energy Corp. Model 21-103 spectrometer), and the recovered ammonium salts were analyzed by ^1H and ^2H NMR spectroscopy (average data of three parallel experiments are summarized in each case).

When the reaction was carried out using lithium deuteride (Alfa Inorganics isotopic purity >98%), instead of lithium hydride, with a 2:1 molar ratio of NH_4^+:LiD, the hydrogen formed in the reaction showed isotopic scrambling. A composition of 66% HD, 21% H_2, and 13% D_2 was found by mass spectrometric analysis.

$$^{+}NH_4{}^{-}O_2CCF_3 + LiD \longrightarrow CF_3CO_2Li + \underbrace{85\%\ NH_3 + 15\%\ NH_2D}_{} + \underbrace{66\%\ HD + 21\%\ H_2 + 13\%\ D_2}_{} \quad (2)$$

At the same time, 85% NH_3 and 15% NH_2D (with small amounts of NHD_2) were formed. When we investigated the recovered, unreacted ammonium salt, it was shown by ^1H and ^2H NMR spectroscopic analysis to contain about 95% NH_4^+ and 5% NH_3D^+ (or any further deuterated ammonium ion).

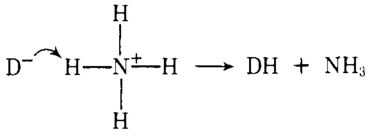
We also carried out the reaction of $^{+}ND_4{}^{-}O_2CCF_3$ with LiH under identical conditions. (The isotopic purity of the ND_4^+ salt was better than 98%, prepared from $ND_4^+Cl^-$ and CF_3CO_2Ag.) In this case, the hydrogen gas composition found was 68% HD, 14% D_2, and 18% H_2, and again ammonia and recovered ammonium ion showed corresponding exchange (eq 3).

$$^{+}ND_4{}^{-}O_2CCF_3 + LiH \longrightarrow CF_3CO_2Li + \underbrace{ND_3 + ND_2H\,(NDH_2)}_{} + \underbrace{68\%\ HD + 14\%\ D_2 + 18\%\ H_2}_{} \quad (3)$$

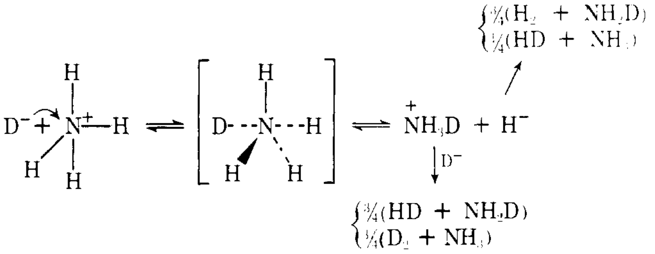
It was rather surprising to observe the significant isotopic scramblings. To ascertain that no impurities, such an unavoidable water (heavy water) was associated with the ammonium salts (which were carefully dried, but still can maintain some absorbed water) or exchange was taking place in the ion source of the mass spectrometer, a series of control experiments were carried out. No scrambling occurred in the ionization chamber as evidenced by the small amount of (<1%) of HD observed on pure samples of D_2 and H_2. Water (or heavy water) impurity in the ammonium ions or any moisture in the system or in contact during analysis could also clearly not account for the extensive scramblings observed. This is particularly true for the formation of H_2 in the $NH_4^+D^-$ and D_2 in the $ND_4^+H^-$ systems. There was, however, scrambling observed in heavy ammonia obtained from the ND_4^+ system, also in the case of $ND_4^+D^-$, indicating it is coming from absorbed water.

Additionally very limited exchange was observed for the LiD + H_2 and LiD + NH_3 + D_2 systems, although in the latter case HD can be formed by the reaction of LiD with NH_3 giving $LiNH_2$. Earlier work reported in the literature on H_2 + D_2 and D_2 + NH_3 showed no isotopic exchange unless a catalyst is present or the temperature is raised to 500–600°.[7] Therefore, all the side reactions discussed can be of only limited significance.

The expected linear type of displacement at the hydrogen atoms of NH_4^+ by lithium deuteride will give HD and ammonia. Thus, no H_2 (or D_2) is expected to be formed in the reaction. However, from the experimental data, it can be seen that this is not the case. The displacement reaction would give only HD and would not account for observed formation of any H_2 or D_2; thus it is not the only reaction taking place. In order to account for the experimentally observed scramblings, including formation of H_2 (and D_2), the most reasonable explanation seems to be that D^- also attacks NH_4^+ on the quaternary nitrogen in an S_N2 like fashion causing exchange to occur via pentacoordinated NH_4D

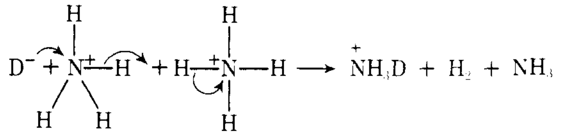


$$D^- \; H-\overset{\displaystyle H}{\underset{\displaystyle H}{\overset{|}{\underset{|}{N^{\pm}}}}}-H \longrightarrow DH + NH_3$$



The deuteriated ammonium ion then can react with hydride (or deuteride) in the linear fashion giving ammonia and hydrogen, appropriately scrambled. The same reactions would also account for the observed isotopic scrambling in case of the $ND_4^+H^-$ system.

Alternatively, in the elimination step from the NH_5 type transition state the leaving of the hydride (deuteride) ion can be assisted by another ammonium molecule



$$D^- + NH_4^+ + H-NH_3^+ \longrightarrow \overset{+}{N}H_3D + H_2 + NH_3$$

This may account for the significant formation of H_2 observed in the reaction, whereas D_2 (and HD) can be formed by attack of D^- on $^{+}NH_3D$.

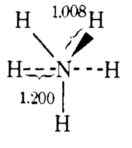
A further possibility, in accordance with a referee's suggestion, would be to consider NH_4D as a real pentacoordinated intermediate which could cleave with possible intervening pseudorotation to give either HD + NH_3 or H_2NH_2D. NH_2D then could pick up a proton from another NH_4^+ to form NH_3D^+ which can lead to the formation of D_2. We have also considered this possibility but, in the case of NH_4D, thought it to be unlikely. After all, one must place ten electrons around nitrogen, similarly to the ten electrons in CH_5^- or related S_N2 systems, where intermediates are unknown. The case of NF_5 (discussed subsequently) may be more favorable due to the high electronegativity of fluorine. For the time being, however, we have no experimental evidence indicating a pentacoordinated nitrogen intermediate. Whether there is, and if so, how shallow a dip on the energy diagram of the exchange reactions following the transition state maximum, can only be speculated upon.

Ammonia and ammonium ion themselves are well known to exchange through a low energy process[8]

$$NH_4^+ + NH_2D \rightleftharpoons [NH_3\cdots H\cdots NH_2D]^+ \rightleftharpoons NH_3 + NH_3{}^{+}D$$

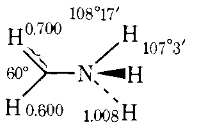
None of the exchange processes, however, could give H_2 from the NH_4^+ + D^- or D_2 from the ND_4^+ + H^- systems, respectively, without involvement of prior nucleophilic hydrogen exchange of the ammonium ion through pentacoordinated NH_5.

Pauling, considering the electronegativities of nitrogen and hydrogen, concluded that the charge distribution in NH_4^+ is about equal over the five atoms.[9] Thus considering the charge effects in the reaction competing attack of the nucleophile on nitrogen seems possible. We also carried out CNDO/2 calculations for the possible limiting D_{3h} and C_s structures of nitrogen pentahydride to minimize the binding energy by varying bond lengths. The calculated heats of formations for these forms are as shown. The trigonal bipyramidal form is favored by some 41 kcal/mol over the C_s symmetry form, as is found in calculations of CH_5^- (in the latter case the larger charge-charge repulsion effect caused a calculated difference of 55 kcal/mol). Further we also calculated the comparative species with hydride attack colinearly on hydrogen



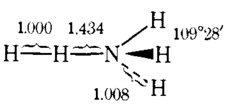
D_{3h}

$\Delta H_f = -613$ kcal/mol



C_s

$\Delta H_f = 572$ kcal/mol

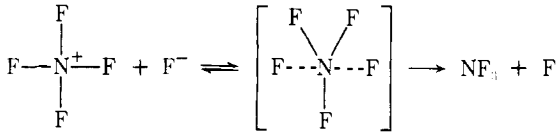


$\Delta H_f = 666$ kcal/mol

The data show that a nucleophilic attack on hydrogen by hydride ion is favored. Even though the calculations seem to favor largely the attack on hydrogen by the hydride ion, the alternative attack on nitrogen cannot be dismissed in view of the experimental facts. One of the possible explanations for the lowering of the barrier in the latter mechanism is the participation of another NH_4^+ molecule which can help the process by providing a proton for the removal of the otherwise unstable displaced hydride ion.

Uncertainties in the geometries used in the calculations and the applicability of the CNDO/2 method to this system could account for the calculated large differences between attack on hydrogen and nitrogen.

In related studies we observed that the known $NF_4^+SbF_6^-$ salt[10] when treated with LiF decomposed to NF_3, F_2, and $LiSbF_6$. This reaction could further indicate the possibility of nucleophilic attack by F^- on nitrogen, as the high electronegativity of fluorine would enhance this mode over attack on fluorine. Whether there is fluorine exchange must await contemplated studies using $^{18}F^-$.



Our present work showing the involvement of pentacoordinated NH_5 in the nucleophilic displacement of the ammonium ion by hydride ion through attack on the quaternary nitrogen opens up of what is considered a new interesting field of nucleophilic displacement reactions on quaternary nitrogen. We are continuing related studies including investigations of such reactions as the nucleophilic displacement of ammonium (including anilinium) ions by nitrite ion, i.e., the nucleophilic analog of the diazotization reaction.

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Solvent Effect on the Magnetic Anisotropy of Low-Spin Dicyano Ferric Complexes of Natural Porphyrins

Sir:

The environment of the prosthetic group in hemoproteins plays a crucial role in modulating the redox and oxygen-binding properties at the central metal ion. The discussion of the spectral and magnetic properties of hemoproteins and their model compounds usually assumes a well-defined electronic structure for the prosthetic group.[1-4] Recent work has shown[2-4] that certain structure-function relationships of hemoproteins can be conveniently studied using the isotropic shift of peripheral porphyrin substituents in the hemoproteins or in model compounds. Although changes in the NMR shifts either within the protein or on going from the model compound to the protein have been interpreted in terms of structural changes in the heme environment,[2-4] to date no explicit recognition has been given to the possible effect of solvation on the electronic structure of the iron. Contrary to earlier indications,[5] we find that the proton NMR, and hence by inference the electronic structure, of low-spin dicyano ferric porphyrins are not only sensitive to the solvent but depend on solvent in a characteristic manner which suggests that solvation should be considered in interpreting changes in the NMR spectra of cyano hemoproteins.[2-4]

Table I presents the chemical shifts[6] for several ring substituents in dicyano complexes of natural porphyrins in various solvents.[7] The shifts change monotonically with solvent, with *all* positions experiencing an overall downfield bias moving up the table. The ring substituent shifts in low-spin ferric porphyrins have been shown to arise from upfield dipolar shifts and π-contact shifts reflecting L → M π charge transfer.[8,9] The changes in shifts with solvent, however, can be shown to reflect primarily changes in the dipolar shift (magnetic anisotropy). This is illustrated in Table II, where the shift changes for various functional groups parallel the calculated relative geometric factors.[8,9] Changes in the ex-